

**From:** [PETERSON Jenn L](#)  
**To:** [Dana Davoli/R10/USEPA/US@EPA](#); [Jay Field](#)  
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**Subject:** RE: Fw: Portland Harbor HH bass composites  
**Date:** 10/02/2007 03:36 PM

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This article has a very good description of the individual and batch method for composites - just click on the link below.

-Jennifer

Title: Procedures for formation of composite samples from segmented populations  
Author: Fabrizio, M.C., A.M. Frank, and J.F. Savino  
TN: 77641

has been received by the Oregon State Library Document Delivery staff.  
You can now  
access this item by clicking on the link below

<http://index.osl.state.or.us/getsmart/pdf/77641.pdf>

-----Original Message-----

From: Davoli.Dana@epamail.epa.gov [mailto:Davoli.Dana@epamail.epa.gov]  
Sent: Tuesday, October 02, 2007 12:32 PM  
To: Jay Field  
Cc: ANDERSON Jim M; audiehuber@ctuir.com; BBarquin@hk-law.com; Benjamin Shorr; Shephard.Burt@epamail.epa.gov; Humphrey.Chip@epamail.epa.gov; csmith@parametrix.com; Blischke.Eric@epamail.epa.gov; erin.madden@gmail.com; Grepo-Grove.Gina@epamail.epa.gov; howp@critfc.org; jeremy\_buck@fws.gov; Goulet.Joe@epamail.epa.gov; LavelleJM@cdm.com; Lisa.Bluelake@grandronde.org; Cora.Lori@epamail.epa.gov; Mary.Baker@noaa.gov; MCCLINCY Matt; Michael.Karnosh@grandronde.org; PETERSON Jenn L; POULSEN Mike; Fuentes.Rene@epamail.epa.gov; rgensemer@parametrix.com; Robert.Neely@noaa.gov; rose@yakama.com; sheila@ridolfi.com; tom@ctsi.nsn.us  
Subject: Re: Fw: Portland Harbor HH bass composites

Jay, this is an explanation as to why we did the entire fish. It's from the Columbia River Study. I know we talked a lot about how to do the composites before finally deciding on this approach.

#### 8.2 GENERAL CONSIDERATIONS FOR PREPARING COMPOSITES

Composite samples may be prepared using two different methods. In the first method (the "individual" method), each individual fish or fish fillet that is to be part of a composite is homogenized separately. Equal weights of each individual fish homogenate are then compiled into a composite and homogenized again. The individual method is designed to provide information on the mean concentration of contamination in fish tissue for the fish population that is being sampled. In the second method (the "batch method"), all of the fillets or whole fish that are to be part of a composite are homogenized together. The batch method provides information on the weighted mean of the concentration in the batch sampled.

For this project, composites will be homogenized by the subcontract laboratory using the batch method. Information on the fish consumption habits of tribal members suggest that once fish are caught, the entire fish is consumed. Therefore, the information on contaminant levels provided by the batch method (which includes information from the entire fillet of each fish in a composite) will provide a more appropriate estimate of exposure for the Native Americans. It is expected that since every attempt will be made to ensure that fish that make up a composite sample will be similar in size (i.e., the smallest individual will be no less than 75% of the total length of the largest individual), the mean concentrations generated by the batch method will likely be similar to that generated using the individual method.

The batch method is also easier to implement in the laboratory because it saves sample preparation time and resources and maximizes the amount of tissue available after grinding smaller fish. This is because tissue from smaller fish often remain inside the grinder due to the small volume of sample going through the grinder.

Jay Field  
<Jay.Field@noaa.gov>

To

10/02/2007 10:42  
AM

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Subject  
Re: Fw: Portland Harbor HH bass  
composites

Dana,  
The guidance specifically recommends using equal amount of homogenate from individual fish to create the composite (see Figure 7-1. Preparation of fish fillet composite homogenate sample and p. 7-15: "Composite homogenates should be prepared from equal weights of individual homogenates."). If the recommended approach is not followed,

you will have unequal sample size in your composites, which will not provide an unbiased estimate of the mean. If the guidance for creating composite samples is followed, I would have no problem using the 0.75 length factor for creating acceptable composites. Note that the guidance also recommends archiving homogenate from each individual fish.  
Jay

Davoli.Dana@epamail.epa.gov wrote:

> Jay, I do not think the guidance recommends using unequal amounts from  
  
> each fish to create the composite. I have included the language from  
the  
> guidance below so you can make your own interpretation. The site is:  
> <http://www.epa.gov/waterscience/fishadvice/volume1/vlch6.pdf>  
>  
>  
> 6.1.1.6 Sample Type  
> (Page 6-18)  
> Note: Composite samples are homogeneous mixtures of samples from two  
or  
> more individual organisms of the same species collected at a  
particular  
> site and  
> analyzed as a single sample. Because the costs of performing  
individual  
> chemical  
> analyses are usually higher than the costs of sample collection and  
> preparation, composite samples are most cost-effective for estimating  
> average  
tissue  
> concentrations of target analytes in target species populations.  
Besides  
> being  
> cost-effective, composite samples also ensure adequate sample mass to  
> allow analyses for all recommended target analytes. A disadvantage of  
> using composite samples, however, is that extreme contaminant  
> concentration values  
> for individual organisms are lost.  
>  
> In screening studies, EPA recommends that states analyze one composite  
sample for each of two target species at each screening site.  
Organisms  
> used in  
> a composite sample:  
> Must all be of the same species  
> Should satisfy any legal requirements of harvestable size or  
weight,  
> or at least

- > be of consumable size if no legal harvest requirements are in effect.
- > Should be of similar size so that the smallest individual in a composite is no less than 75 percent of the total length (size) of the largest individual. Should be collected at the same time (i.e., collected as close to the same time as possible but no more than 1 week apart)
- [Note:
  - > This assumes that a sampling crew was unable to collect all fish needed to prepare the composite sample on the same day. If organisms used in the same composite are collected on different days (no more than 1 week apart),
- > they should be
  - > processed within 24 hours as described in Section 7.2 except that individual fish may have to be filleted and frozen until all the fish to be included in the composite are delivered to the laboratory. At that time, the composite homogenate sample may be prepared.]
  - > Should be collected in sufficient numbers to provide a 200-g composite homogenate sample of edible tissue for analysis of recommended target analytes.
- > Individual organisms used in composite samples must be of the same species because of the significant species-specific bioaccumulation potential. Accurate taxonomic identification is essential in preventing the mixing of closely related species with the target species. Note: Individuals from different species should not be used in a single composite sample (U.S. EPA, 1989d, 1990d).
- > For cost-effectiveness, EPA recommends that states collect only one size class for each target species and focus on the larger individuals commonly harvested by the local population. Ideally, each composite sample for a specific species should contain the same number of individual fish and the individuals within each target species composite should be of similar size within a target size range so that the composite samples for a particular species are comparable over a wide geographic area. This is particularly important when states want to compare data on an individual species that might be used to establish a statewide advisory.
- > For persistent chlorinated organic compounds (e.g., DDT, dioxin, PCBs, and toxaphene) and methylmercury, the larger (older) individuals within a population are generally the most contaminated (Phillips, 1980; Voiland et al., 1991). As noted earlier, this correlation between increasing size and increasing contaminant concentration is most striking in freshwater finfish species but is less evident in estuarine and marine species. Size is used as a surrogate for age, which provides some estimate of the total time the individual organism has been at risk of exposure. Therefore, the primary target size range ideally should include the larger individuals harvested at each sampling site. In this way, the states will maximize their chances of detecting high levels of chemical contamination in the single composite sample collected for each target species. If this ideal condition cannot be met, the field sampling team should retain individuals of similar length that fall within a secondary target size range.
- > Individual organisms used in composite samples should be of similar size
  - > (WDNR, 1988). Note: Ideally, for fish or shellfish, the total length (or size) of the smallest individual in any composite sample should be no less than 75 percent of the total length (or size) of the largest individual in the composite sample (U.S. EPA, 1990d). For example, if the largest fish is 200 mm, then the smallest individual included in the composite sample should be at least 150 mm.
- > In the California Mussel Watch Program, a predetermined size range (55 to 65 mm) for the target bivalves (*Mytilus californianus* and *M. edulis*) is used as a sample selection criterion at all sampling sites to reduce size-related variability (Phillips, 1988).
- > Similarly, the Texas Water Commission (1990) specifies the target size range for

- > each of the recommended target fish species collected in the state's
- > fish
- > contaminant monitoring program.
- >
- > Individual organisms used in a composite sample ideally should be
- > collected at the same time so that temporal changes in contaminant
- > concentrations associated with the reproduction cycle of the target
- > species are minimized.
- >
- > Each composite sample should contain 200 g of tissue so that
- sufficient
- > material
- > will be available for the analysis of all recommended target analytes.
- A
- > larger
- > composite sample mass may be required when the number of target
- analytes
- > is
- > increased to address regional or site-specific concerns. However, the
- > tissue mass may be reduced in the Tier 2 intensive studies (Phase I
- > and II) when a
- > limited number of specific analytes of concern have been identified
- (see
- > Section
- > 7.2.2.9). Given the variability in size among target species, only
- > approximate ranges can be suggested for the number of individual
- > organisms to collect to
- > achieve adequate mass in screening studies (U.S. EPA, 1989d; Versar,
- > 1982).
- > For fish, 3 to 10 individuals should be collected for a composite
- sample
- > for each
- > target species; for shellfish, 3 to 50 individuals should be collected
- >
- > for a composite sample. In some cases, however, more than 50 small
- > shellfish (e.g., mussels,
- > shrimp, crayfish) may be needed to obtain the recommended 200-g sample
- > mass.
- > Note: The same number of individuals should be used in each composite
- > sample
- > for a given target species at each sampling site.
- >
- > Deviations from the recommended study design have implications that
- may
- > make
- > the statistical analyses more complicated. The statistical methods for
- >
- > analyzing composite samples are made tractable and easier-to-use by
- > simplifying the study
- > design. Using equal numbers of fish in replicate composite samples is
- > one way
- > to do this. For example, with equal numbers of fish, the arithmetic
- > average of the
- > replicate composite measurements is an unbiased estimator of the
- > population
- > mean. When unequal numbers are used, the arithmetic average is no
- longer
- > unbiased. Instead, a weighted average of the composite measurements is
- >
- > calculated, where the weight for each composite reflects the number of
- >
- > fish it is made up of. Oftentimes fish are lost or damaged prior to
- > compositing. When
- > several fish are damaged or lost, the allocation of the remaining fish
- > to
- > composites may be reconfigured to allow equal numbers of fish in
- > composites. If
- > this is not possible, care should be taken to adjust the statistical
- > procedures to
- > account for the unequal allocations.
- >
- > The use of sizes of fish exceeding the size range recommended for
- > compositing may introduce more variability. If it is the size range
- > within each composite that
- > is broadened (e.g., 100-200 mm instead of 150-200 mm), the variability
- > within the
- > composite may increase. If additional composites are made with fish
- > exceeding
- > the recommended size ranges (e.g., adding composites of fish of size
- > 300-450
- > mm when the target size is no more than 250 mm), this may increase the
- > variability between composites of different size ranges. Overall
- > inferences made
- > from composites of different size ranges will have increased
- variability
- > associated
- > with them (e.g., wider confidence intervals).
- >
- > Differences in the numbers of replicates at different sampling
- locations
- > may
- > complicate any comparisons to be made between locations or overall
- > conclusions to be obtained by combining the results from different
- > sampling locations. As with
- > unequal numbers of fish in composites, unequal numbers of replicate
- > samples
- > complicate the statistical calculations. The appropriate weighted
- > estimates should
- > be used when combining information from different sampling locations.
- > Consider,
- > for instance, a state that monitors five lakes each year. If the state

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> uses the same
> target fish species, the same number of fish per composite and the
same
> size
> ranges, the overall mean level of contamination will be a
> straightforward average over the five locations if the same number of
> replicates are used at each location.
> However, if unequal numbers of replicates are used, the information
> contributed
> by each location is not the same and must be weighted accordingly.
>
> As alluded to above, one limitation of using composite samples is that
> information on extreme levels of chemical contamination in individual
> organisms is lost.
> Therefore, EPA recommends that the residual individual homogenates be
> saved
> to allow for analyses of individual specimens if resources permit
> (Versar, 1982).
> Analysis of individual homogenates allows states to estimate the
> underlying
> population variance which, as described in Section 6.1.2.6,
facilitates
> sample size
> determination for the intensive studies. Furthermore, individual
> homogenates may also be used to provide materials for split and spike
> samples for routine QC
> procedures either for composites or individual organisms (see Section
> 8.3). The
> circumstances in which the analysis of individual fish samples might
be
> preferred
> over the analysis of composite samples is described in more detail in
> Appendix C. Recommended sample preparation procedures are discussed in
>
> Section 7.2 .
>
>
>
> Jay Field
>
> <Jay.Field@noaa.
gov>
To Dana Davoli/R10/USEPA/US@EPA
>
> 10/02/2007 09:43
cc AM
>
> Subject Re: Fw: Portland Harbor HH bass
composites
>
>
>
>
>
>
>
>
>
>
> Dana,
> does the guidance recommend using unequal amounts from each fish to
> create the composite? Jay
>
> Davoli.Dana@epamail.epa.gov wrote:
> Jay, for the PH RI Round 1 and this round of sampling we have
been
> following the guidance given in USEPA "Guidance for Assessing
> Chemical
> Contaminant Data for Use in Fish Advisories". This guidance
> recommends
> using the 0.75 length criteria for composites. We are using the
> entire
> fish, not an aliquot. Sex of the fish has not been considered.
> Thanks!
>
>
>
>
> Jay Field
>
> <Jay.Field@noaa.
gov>
To Dana
```

[illegible]



> There are three objectives to collecting bass composites  
 > during Round  
 > 3B:  
 >  
 > \* Estimating risks to human health from  
 > consumption of fish  
 > \* Estimating risks to ecological receptors, and  
 > assisting  
 > with  
 > refinement of the foodweb model  
 > \* Identifying sources by identifying the  
 presence  
 > of  
 > chemicals in  
 > fish from separate reaches of the river  
 >  
 > We think that the compositing method proposed will meet  
 the  
 > human  
 >  
 > health  
 >  
 > risk assessment objective. However, we are seeking  
 comments  
 > from the  
 > rest of the EPA team on whether this approach is  
 appropriate  
 > to meet  
 >  
 > the  
 >  
 > other objectives.  
 >  
 > I took LWG's R3B\_bass-carp\_lengths.xls spreadsheet,  
 deleted  
 > carp data,  
 > deleted some columns on weight, and added columns  
 > summarizing the  
 > composites. For each river-mile portion (by bank), I  
 sorted  
 > by fish  
 > length. The five longest fish are included in the proposed  
 > composites.  
 > This is indicated by an "x" in the column next to the  
 > length.  
 >  
 > For comparison, data from Round 1 bass are included at the  
 > bottom of  
 >  
 > the  
 >  
 > spreadsheet. Overall, the fish collected in Round 3 are  
 > similar in  
 >  
 > size  
 >  
 > to the fish collected in Round 1. There were some large  
 fish  
 > caught  
 >  
 > and  
 >  
 > released in Round 3 because they were substantially larger  
 > than the  
 > planned limit of 355 mm. There were four fish greater than  
 > the maximum  
 > Round 1 length of 430 mm, with a maximum length of 530 mm.  
 I  
 > do not  
 > think that omitting the released fish will have a  
 > substantial effect  
 >  
 > on  
 >  
 > the results of the chemical analyses.  
 >  
 > Our original criteria for including fish in a composite  
 were  
 > lengths  
 > between 225 mm and 355 mm, and a ratio of smallest fish in  
 > composite  
 >  
 > to  
 >  
 > largest fish of 0.75 or greater. This was to avoid a  
 > situation where  
 >  
 > one  
 >  
 > large fish would dominate the concentration in a  
 composite,  
 > and to  
 > minimize size as a variable that needs to be considered in  
 > evaluating  
 > the data. Fish that are longer generally weigh more, and  
 are  
 > generally  
 > older than smaller fish. Older fish are more likely to  
 have  
 >  
 > accumulated



> chemicals of interest. Larger fish are more desirable as  
 > food fish.  
 >  
 > For  
 > these reasons, including larger fish in the composite  
 > meets the needs  
 >  
 > of  
 > the human health risk assessment. However, we understand  
 > that larger  
 > fish may not be appropriate for the ecological risk  
 > assessment.  
 >  
 > The selection criteria were not strictly applied in Round  
 1.  
 > Many of  
 >  
 > the  
 > fish included in composites were greater than 355 mm. The  
 > criterion of  
 > 0.75 was not always met. EPA and LWG accepted the  
 composite  
 > approach  
 >  
 > in  
 > Round 1, acknowledging that not all the criteria were met.  
 > We  
 > therefore  
 > do not feel strictly bound by the criteria in Round 3.  
 > Using the proposed compositing approach, four of the  
 reaches  
 > do not  
 >  
 > meet  
 >  
 > the 0.75 criterion: RM 6 East (0.74), RM 6 West (0.71), RM  
 8  
 > West  
 > (0.70), and RM 10 West (0.64). If the maximum length of  
 403  
 > mm is  
 > removed from RM 10 West and replaced with the 251 mm  
 value,  
 > the  
 >  
 > revised  
 >  
 > ratio is 0.77.  
 >  
 > In Round 1, the mean length in a composite was generally  
 > less than 300  
 > mm. In Round 3, the mean length is generally greater than  
 > 300 mm,  
 > particularly in upstream sampling areas. The two areas  
 with  
 > the  
 >  
 > largest  
 >  
 > difference between sides of the river are RM 6 (271 mm  
 East  
 > v. 315 mm  
 > West) and RM 11 (271 mm East v. 338 mm West). It is not  
 > clear if  
 > differences of this size in fish would confound  
 comparisons  
 > of areas.  
 > For fish of similar sizes, differences in concentrations  
 may  
 > be  
 >  
 > related  
 >  
 > to proximity to source areas. However, if one of the  
 reasons  
 > for the  
 > differences in concentrations is the size (age) of fish,  
 > this could  
 > confound a determination of sources.  
 >  
 > - Mike  
 > <<HH bass composites R3B.xls>>  
 > (See attached file: HH bass composites R3B.xls)  
 >  
 >  
 >  
 > --  
 > Jay Field  
 > Assessment and Restoration Division  
 > Office of Response and Restoration, NOAA  
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